

although there seemed to be a trend between ACE genotypes and longevity, it was not significant. No such trend was found in eNOS or PON1 polymorphisms. Further studies with larger numbers are needed to determine if common variants in genes associated with cardiovascular risk contribute significantly to longevity.

PP-954

A study on the interaction between β lactoglobulin-A and a new antitumor reagent (2,2-bipyridinglycinato Pd (ii) chloride)

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Bovine β lactoglobulin (Blg) is a major whey protein of bovine milk. The physiological function of Blg is considered to be the binding and transportation of small hydrophobic ligands. The interaction between a new Pd (II) complex, as an antitumor component, with β lactoglobulin was studied using fluorescence spectroscopy and Far UV-CD spectropolarimetric techniques. A strong fluorescence quenching reaction of Pd (II) complex with Blg-A was observed and the quenching mechanism was suggested as a static quenching mechanism. The binding constants of Pd (II) complex with Blg-A at different temperatures of 300, 310, 315 and 320 K were calculated as 390, 420, 660 and 630/nM and corresponding the average numbers of binding sites were 4.4, 3.3, 3.2 and 3.5, respectively. Thermodynamic parameters calculated at different temperatures indicate that the hydrophobic forces play a major role in the interaction of this complex to Blg-A. Far-UV-CD studies showed that in the presence of different concentrations of Pd (II) complex, the secondary structure of the protein does not any significant change at different temperatures. From above results, it can be seen that Pd (II) complex can significantly change the tertiary structure of Blg-A, without any effects on the secondary structure of the protein at different temperatures. The new Pd (II) complex can bind to Blg-A to be effectively transported and eliminated in body, which can be a useful guideline for further drug design.

PP-955

A comparative study on the effect of less moisture solvents on solubilized lactase phlorizin hydrolase

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The study of enzymes in less moisture solvents is of great importance. Increasing number of reports has been appeared regarding to the pure soluble enzymes under such conditions. Meanwhile there are few reports mirroring the effect of minimally moist solvent systems on membrane bound enzymes. Lactase-phlorizin hydrolase (LPH; EC 3.2.1.23/62) is a membrane bound intestinal hydrolase, with an extracellular domain comprising four homologous regions. It is synthesized as a large polypeptide precursor, pro-LPH that undergoes several intra- and extracellular proteolytic steps to generate the mature membrane bound enzyme which have been emphasized in this study in both in situ and detergent mediated solubilized form. The later type of the enzyme was prepared upon Triton X-114 phase partitioning technique. So, we separated LPH from residual intestinal brush border

membrane (IBBM) proteins based on its hydrophobic characteristics which resulted in enriched LPH in detergent poor phase (DPP). In this report the effect of type and percent of water miscible organic solvents in three categories; polar-protic, polar-aprotic and nonpolar-aprotic solvents, are examined on the stability and activity of two mentioned preparations of LPH (IBBM and DPP). Our results indicate acceptable catalytic properties of the LPH in less moisture systems. Furthermore the relative activation of the LPH was observed in nonpolar-aprotic solvents, especially for solubilized LPH.

PP-956

Nickel ions induced substrate inhibition for DAAO

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The effect of Ni ions on the structure and activity of D-amino acid oxidase (DAAO) was investigated at pyrophosphate buffer, 37 Centigrade. The activation and inhibitory concentration ranges of Ni (II) on DAAO were also determined. Ni ions at low concentrations (<2 mM) induced substrate inhibition for DAAO at high concentrations of Alanine as a main substrate accompanying with compaction of the enzyme which was observed by Far-circular dichroism (CD) experiment.

PP-957

Improving the purification of NAD⁺-dependent formate dehydrogenase from *Candida methylica*

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One important use of formate dehydrogenase (FDH) is to regenerate valuable NADH which is required by NAD⁺-dependent oxidoreductases (e.g. LDH) in enzymic catalysis. The NAD⁺-dependent FDH offers several advantages over any of the other dehydrogenases, and has been extensively studied as a candidate for developing industrial NADH regeneration. The advantages of using FDH are; the availability and low cost, a favourable thermodynamic equilibrium and the inertness of the CO₂ product. On the other hand the low *k*_{cat}, and high *K*_M, the lack of extreme thermostability or the solvent tolerance and the limited coenzyme specificity are known disadvantages of FDH. In order to make FDH more thermostable enzyme for its industrial applications, NAD⁺-dependent FDH from *Candida methylica* will be engineered by using site directed mutagenesis. To be able to purify each constructed mutant protein more efficiently, *Candida methylica* recombinant wild type FDH gene was cloned into the pQE-2 TAGZyme expression vector and 6his-tagged FDH gene was overexpressed in JM105 cells. By the use of exopeptidases, TAGZyme Purification System allowed the complete removal of the small N-terminal His tag. 1.064 mg/ml cmFDH protein of >95% purity was obtained after the purification procedure. The